DEVELOPMENTAL BIOLOGY 175, 248–255 (1996)
ARTICLE NO. 0112

Epidermal Fate Map of the *Arabidopsis*

Arp Schnittger,* Paul E. Grini,*,† Ulrike Folkers,* and Martin Hülskamp*,[1]

Lehrstuhl für Entwicklungsgenetik, Spemannstrasse 37-39, 72076 Tübingen, Germany; and †Department of General Genetics, Biology Institute, University of Oslo, P.O. Box 1031 Blindern, 0315 Oslo, Norway

The shoot meristem generates all of the aerial structures of an adult plant. It is organized in three layers which produce the epidermis (L1 layer) and the subepidermal layers (L2 and L3). The origin of adult structures has previously been fate mapped to the primary meristem for L2 and L3 tissues. In this work we constructed a fate map of L1 cells in the embryonic shoot meristem. Using the trichome mutation *stichel* as an epidermal marker, we analyzed 153 plants that included 178 sectors. Sectors on early leaves were found to be smaller and occurred more frequently than those on late leaves. Sectors on late leaves also appeared often to affect more than one leaf. In general, the width and extent of sectors were found to be variable rather than cell lineage-restricted. Our analysis allowed us to assign the most likely fates of L1 precursor cells within the embryonic shoot meristem. The results suggest that the meristem integrates growth dynamics and patterning of all three tissues. In contrast to this coordinated growth behavior of meristematic cells, we found a difference in the lineage restrictions between the L1 and the L2 for the formation of axillary buds.

INTRODUCTION

During plant embryogenesis two groups of stem cells are organized: the primary shoot and the root meristems. The shoot meristem generates all aerial structures of the adult plant. How is the development of adult structures linked to the organization in the embryonic shoot meristem? Is the cell fate of adult structures already laid down in the embryonic shoot meristem or does the meristem acquire its positional information later in development? These questions have been addressed by studying the cell fate of meristematic cells through genetic labeling of single cells in the shoot meristem. This way fate maps of the primary shoot meristem have been constructed for different plant species including maize (Steffensen, 1968; Coe and Neuffer, 1978; Johri and Coe, 1983; McDaniel and Poethig, 1988), sunflower (Jegla and Sussex, 1989), tobacco (Poethig and Sussex, 1985a, 1985b) and *Arabidopsis* (Furner and Pumfrey, 1992; Irish and Sussex, 1992). Overall the previous data suggest that the fate of a given cell in the shoot meristem is not absolutely determined. However, it is predictable in a more general way. Two parameters appear to affect the cell fate of the shoot meristem (Poethig, 1987; Sussex, 1989; Irish, 1993): One parameter is the growth dynamic of the proliferating meristem. A meristem can be considered to consist of outer and inner ranks of cells. During development outer ranks of cells become recruited for the initiation of organs, leaving progressively fewer cells for later structures. The second parameter is the generation of positional information for the initiation of primordia. Thus the combination of the growth dynamics and the mechanism of pattern formation appears to determine the fates of cells in the primary meristem.

However, the cellular organization of the meristem presents additional complexity. The analysis of single layer periclinal chimeras (cells are differentially marked in each meristematic layer) demonstrated that meristems of dicotyledonous plants are arranged in three distinct single cell layers (Satina *et al.*, 1940). The outermost layer, L1, divides anticlinally (the division plane is perpendicular to the embryo surface) and gives rise to the epidermis. The inner layers L2 and L3 produce the ground tissues and the core tissues, respectively. Thus the three layers reflect the radial organization in the adult plant. The establishment of this pattern can be traced back to the globular stage of embryogenesis. A number of coordinated periclinal divisions

[1] To whom correspondence should be addressed. Fax: -49-7071-601862. E-mail: mh@fserv1.mpib-tuebingen.mpg.de.

0012-1606/96 $18.00

(division plane is parallel to the embryo surface) give rise to the dermatogen, the future epidermis (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994). The inner cells differentiate to form a subepidermal layer of ground tissue and an inner group of vascular precursor cells. The incipient shoot meristem arises from these three layers at the torpedo stage (Barton and Poethig, 1993).

The analysis of periclinal chimeras revealed that the three different layers proliferate through preponderant anticlinal planes of division, thereby maintaining three independent sheets of tissues (Stewart and Burk, 1970). Interactions between the three layers appear to play an important role for morphogenetic events. It has been shown that either layer can have an inductive influence on one or more of the other layers (Stewart *et al.*, 1972; Szymkowiak and Sussex, 1989, 1992; Carpenter and Coen, 1990).

Thus, previous data indicate that the three different layers are formed in a clonally independent fashion and function independently to elicit morphogenetic events. We would like to understand whether these differences are reflected in cell fate differences of the different layers. We compare our fate map for the L1 layer with previous data reported for the L2 layer in *Arabidopsis* (Furner and Pumfrey, 1992; Irish and Sussex, 1992). In this report we present our study of the fate of epidermal cells in the dry seed shoot meristem of *Arabidopsis.* Using the trichome mutation *stichel* as an epidermal marker, we construct a fate map of the primary shoot meristem.

MATERIALS AND METHODS

Arabidopsis seeds of the ecotype Landsberg *erecta* that were homozygous mutants for *Triptychon* (*Try*) and heterozygous for *stichel* (*sti*) were treated with an aqueous solution of 0.3% (v/v) ethylmethanesulfonate (EMS) for 8 hr. In order to obtain a more uniform penetration of the mutagen, the seeds were cold-treated for 4 days at 4°C and redried prior to the EMS treatment (Koornneef *et al.,* 1982; Mayer *et al.,* 1991). After EMS treatment, the seeds were washed thoroughly and sown immediately in 5-cm pots with a density of 30 plants per pot. Plants were grown at 25°C under constant illumination. Pots found to contain one or more plants with a *sti* sector were kept for further analysis. Plants without *sti* sectors were removed from these pots and plants with sectors were monitored for position and size of sectors.

RESULTS

Induction and Phenotype of stichel Sectors

We used the trichome mutation *sti* as a convenient marker to score for epidermal sectors. Trichomes are specialized cells that develop from single epidermal cells early in leaf development (Uphof, 1962). In *Arabidopsis,* trichomes typically form three branches on rosette leaves (Hülskamp *et al.,* 1994; Fig. 1A). The *sti* allele used in this study results in a lack of branching (Fig. 1C). On wild-type cauline leaves trichomes generally show a reduced number of branches. In order to also allow the unambiguous identification of *sti* sectors on cauline leaves, we utilized the *Try* homozygous background. This mutation results in an increased trichome cell size and a larger number of branches (Hülskamp *et al.,* 1994; Fig. 1B).

The strategy to induce epidermal sectors was to treat seeds heterozygous for the trichome mutant *stichel* with the chemical ethylmethanesulfonate (EMS). A mutation in the wild-type *sti* allele is expected to result in a cell that is homozygous for *sti.* If this cell contributes to vegetative tissues it will give rise to a *sti* sector.

Trichomes are evenly distributed on rosette and cauline leaves. Under our growth conditions, the first two rosette leaves carry approximately 10–15 trichomes while later leaves and cauline leaves have approximately 40 trichomes. We estimated that the trichome distribution and density on leaf 3 and later leaves allow the detection of sectors constituting about 10% or more of the leaf area.

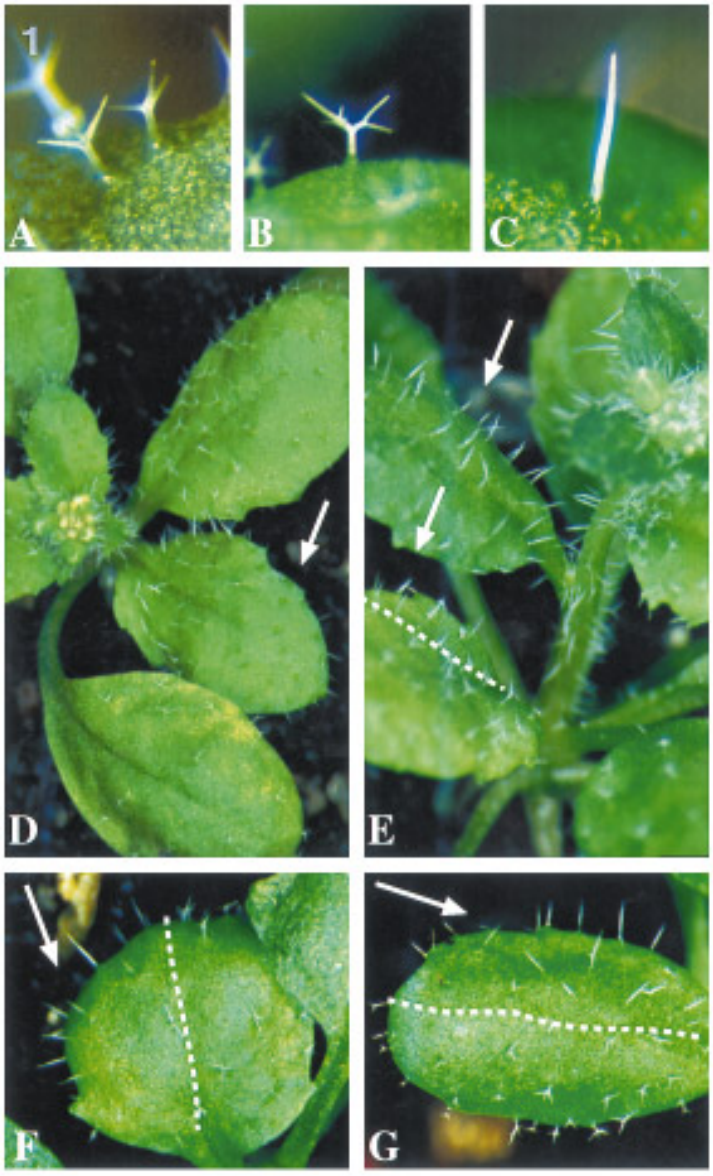
Of 245,000 seeds that were treated with 0.3% EMS approximately 165,000 germinated. *sti* sectors were recovered from about 0.1–0.2% of all plants heterozygous for *sti.* We did not find sectors of any other trichome genes, suggesting that the *sti* sectors found indeed result from a mutation in the wild-type *sti* allele. This view is supported by the finding that of 62 chimeric plants, all plants were heterozygous for *sti.*

Distribution of Sectors

sti sectors were found on 153 plants. Of these mosaic plants about 10% (18 plants) showed sectors affecting more than one leaf. In general, more sectors were found for early leaves than late leaves (Table 1). Sectors on leaf 8 and in the inflorescence were three to four times less frequent than on leaves 3 and 4. Because of the intrinsic lower density of trichomes on the first two leaves, we corrected for the number of sectors by multiplying the number found by the difference in trichome density. However, due to the smaller average size of sectors on early leaves, we believe that our corrected numbers still represent an underestimate (Table 1).

In general, the average sizes of sectors progressively increase in later leaves. While sectors on leaves 3 and 4, on average, contribute to one-third of the leaf, late leaves typically have larger sectors of, on average, more than 50% of the leaf area (Table 1). In addition, sectors that affect late leaves often span more than one leaf (50%). Sectors on early leaves only occasionally contribute to more than one leaf (10%) (Table 1, Fig. 1E).

Taken together these data suggest that the number of precursor cells at the time of induction is different for early leaves than for late leaves. Assuming that all precursor cells are equally sensitive to the mutagenesis, the frequency of sectors for individual leaves reflects the number of its initials. However, in order to take into account the large num-



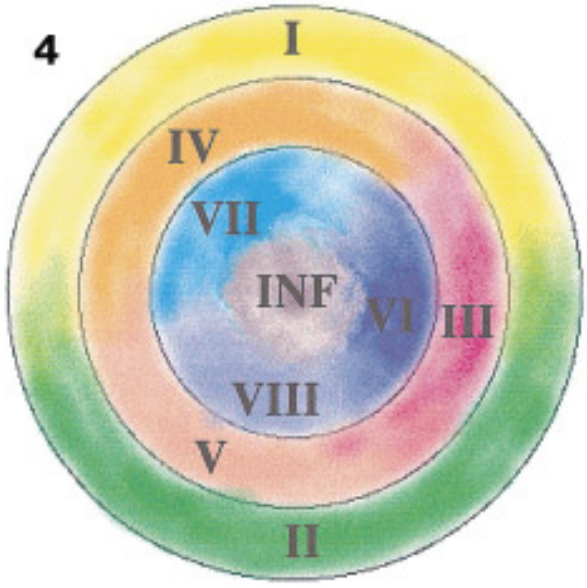


FIG. 1. *sti* sectors on rosette and cauline leaves of *Arabidopsis.* (A) Wild-type trichomes. (B) *Triptychon* trichome. (C) *stichel* trichome. (D) *stichel* sector affecting a whole leaf (arrow). (E) *stichel* sector at the margin of a rosette leaf that continues into the inflorescence (see arrows). (F) *stichel* sector covering the entire left half of a rosette leaf (clonal boundary indicated by a dotted line). (G) *stichel* sector that affects the right half leaf of a cauline leaf (clonal boundary indicated by a dotted line).

FIG. 4. Probability map of the epidermal layer of the dry seed shoot meristem in *Arabidopsis.* The regions of the meristem that most likely contribute to particular vegetative tissues (leaves 1–8 and cauline leaves of the inflorescence (INF)) are labeled correspondingly.

ber of multileaf sectors for late structures, the number of sectors was corrected by dividing the number of sectors by the average number of structures affected. In this way, marked cells are mathematically considered to contribute to only one structure. The number of precursor cells for each of the structures in the dry seed shoot meristem was calculated by multiplying the relative frequency of sectors by the number of L1 cells in the meristem (as estimated by Irish and Sussex, 1992). We calculated that early leaves arise from approximately seven or eight precursor cells while late structures originate from only one or two precursor cells (Table 1).

These findings fit with our analysis on the size, shape, and relative position of sectors on individual leaves. If many cells contribute to a leaf primordium, one would expect small and narrow sectors with a position anywhere on the

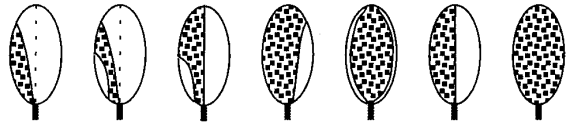
TABLE 1
Frequency of Leaf Sectors in Rosette Leaves and in the Inflorescence

	Leaf number									
	I[a]	II[a]	III	IV	V	VI	VII	VIII	INF	Total
Number of sectors[b]	10 (40)	11 (44)	39	35	27	17	16	10	13	178
% leaf area[c]			36	35	52	36	51	52	70	
Average number of structures affected per plant	(1)	(1)	1.2	1.2	1.4	1.3	1.3	2.3	2.4	
Number of sectors divided by the average number of structures affected	(40)	(44)	32	29	19	13	12	4	5	198
Frequency in %[d]	(20)	(22)	16	15	10	6	6	2.5	2.5	100
Estimated number of L1 cells for each primordium[e]	(7)	(8)	6	6	4	2	2	1	1	37

[a] The numbers in brackets show the corrected number of sectors. Due to the smaller number of trichomes on leaves 1 and 2 the number of found sectors was multiplied by four to compensate for the difference between the first two leaves and the later leaves.
[b] Multileaf sectors were included as occurring on each of the leaves affected.
[c] Calculated from the estimated fraction of the individual clones expressed in percent of the leaf area.
[d] Calculated from the number of sectors divided by the average number of structures affected.
[e] Calculated by multiplying the number of L1 cells in the dry seed stage shoot apical meristem (as estimated by Irish and Sussex, 1992) with the frequency of sectors.

leaf. In contrast, if the meristem at the time of leaf primordium formation is only populated by two precursor cells, sectors should cover whole or large regions of the leaf starting at the leaf margins. In the latter scenario sector borders are simply determined by the relative position of the marked cells with respect to the phyllotactic subdivision of the meristem. As shown in Fig. 2, small sectors that are not attached to the leaf margins were found only on early leaves. In contrast, sectors on late structures such as leaf 8 and leaves from the inflorescence were typically large and always originated from the leaf margin.

A surprisingly large number of sectors terminated exactly at the midvein (Fig. 2). This observation can be explained either by cell lineage restrictions or by the division patterns of the leaf primordia. Since we found a few sectors that crossed the midvein, it appears unlikely that these observations reflect a strict cell lineage restriction. We suggest that the strong correlation of clonal boundaries with a morpho-



								total for each leaf
leaf III	30	13	5	15	8	15	3	39
leaf IV	40	3	14	11	23	9		35
leaf V	22		4	15	7	41		27
leaf VI	35		12	12	6	35		17
leaf VII	13	7	13	13	13	33	7	16
leaf VIII	60			20			2	10
cauline leaves	15			9	3	47	26	34

FIG. 2. Classification of clonal boundaries. Sectors were classified with respect to shape, relative position, and size for each of the rosette (leaves 1–8) and cauline leaves of the inflorescence. The frequency for the different categories are shown as a percentage of the total number of sectors found for each leaf.

logical boundary probably reflects the cell division patterns during leaf blade expansion. This view is supported by the finding that all sectors found extend from the base to the tip of a given leaf, suggesting that initial cell files generate equal portions of the mature leaf by a regular pattern of cell division (Poethig and Sussex, 1985b; Dolan and Poethig, 1991).

Probability Map of the Primary Shoot Meristem

In principle, fate maps can be constructed by calculating the probability that any two structures in the adult body contain tissues derived from the same clonal sector induced in the embryo (Sturtevant, 1929; Hotta and Benzer, 1972). Analogously, we deduced the relative position of precursor cells for individual structures in the primary shoot meristem from plants displaying sectors affecting more than one leaf. The rationale behind this is that multileaf sectors reflect border regions of organ primordia in the primary shoot meristem (Furner and Pumfrey, 1992; Irish and Sussex, 1992).

We found 18 plants with multileaf sectors affecting two to four leaves (Fig. 3A). Almost all sectors affecting the inflorescence were multileaf sectors (see Fig. 3B for examples); only one plant showed a sector that affected the inflorescence exclusively. This indicates that floral tissues cannot be fate mapped as arising from single cells in the primary shoot meristem. They are rather recruited from nearby cell populations that give rise to late leaves.

A fate map of precursor cells of the primary shoot meristem was constructed by combining different sets of data. Figure 4 shows a schematic illustration of the relative contribution and position of organ primordia in the epidermal shoot apex of *Arabidopsis.* In this model, the cells are arranged in three ranks. To account for the different numbers of primordia cells (Table 1), the size of the primordia was drawn proportional to the relative contribution. The relative position of primordia was deduced from our observations of multileaf sectors. In order to illustrate that a given meristem cell will contribute to a particular organ only in a probabilistic way, borders between primordia are drawn to overlap substantially. As suggested previously by Irish and Sussex (1992), we will refer to this type of fate map as a ''probability map'' in the following.

Axillary Meristems

Both rosette leaves and cauline leaves are associated with axillary meristems. To study the origin of the axillary meristems, plants carrying vegetative sectors were decapitated to induce bud outgrowth of these meristems. The relationships between the leaf region affected and the phenotypes of the associated side branch are summarized in Fig. 5. Both sectors at the edge of a leaf and sectors in the center of the leaf were found to be associated with axillary buds bearing mosaic or *stichel* phenotypes. Thus there was no clear correlation between the region affected on the subtending leaf and the phenotype of the side branch.

DISCUSSION

Fate maps have been constructed for various plant species to relate the primary meristem cells to their final contribution in the adult plant. Due to the lack of suitable epidermal markers, previous studies focused on the fate mapping of the L2 and L3 layers of the meristem. In this study we used the trichome mutation *stichel* as an epidermal marker to construct an epidermal fate map of the L1 layer in *Arabidopsis thaliana.*

Most fate maps are based on the genetic labeling of a single cell via the induction of somatic mutations by ionizing radiation or chemical mutagens (Poethig, 1987). However, either of these treatments may bias the distribution and size of mutant sectors. Although ionizing radiation allows the induction of sectors at a high frequency without affecting the germination and growth rate of the treated seeds, it has been demonstrated that the sensitivity of cells to irradiation critically depends on the cell cycle phase (Scott and Evans, 1967; Gudkov and Grodzinsky, 1982; Kowyama *et al.*, 1984). Moreover, ionizing radiation results in the induction of somatic recombination and/or the deletion of chromatids (Poethig, 1987) often associated with growth retardation of the cells affected. In contrast, the application of chemical mutagens such as EMS results in a reduced germination rate possibly due to a high degree of cell death, which in turn favors large sectors through diplontic selection (Gaul, 1959, 1961). In order to minimize such nonspecific damage we worked with low doses of EMS and a short treatment time. Thus the obtained sector frequency and sector distribution of *stichel* probably reflects a realistic picture of the growth dynamics and patterning in the L1 layer in the primary shoot meristem.

The likely fates of meristematic cells can be inferred from the width, length, and distribution of sectors. The width and length of sectors reflect the growth dynamics of the primary meristem, whereas the distribution of multileaf sectors allows a prediction of the relative position of meristematic precursor cells.

The growth dynamics of L1 cells in the shoot meristem appears to be very similar to that observed for L2 markers (Furner and Pumfrey, 1992; Irish and Sussex, 1992). Early leaves tend to have sectors that are small and restricted to only one leaf. Sectors on late leaves are usually larger and often encompass up to several leaves. Our data are consistent with the idea that epidermal cells of the primary meristem are gradually recruited to the flanks of the meristem where they acquire more restricted fates. Thus the center of the meristem contains progressively fewer cells from the primary meristem. This view has an important implication for the interpretation of such fate maps since the apparent cell fate reflects the growth dynamics of the meristem over time as opposed to real cell fate restrictions.

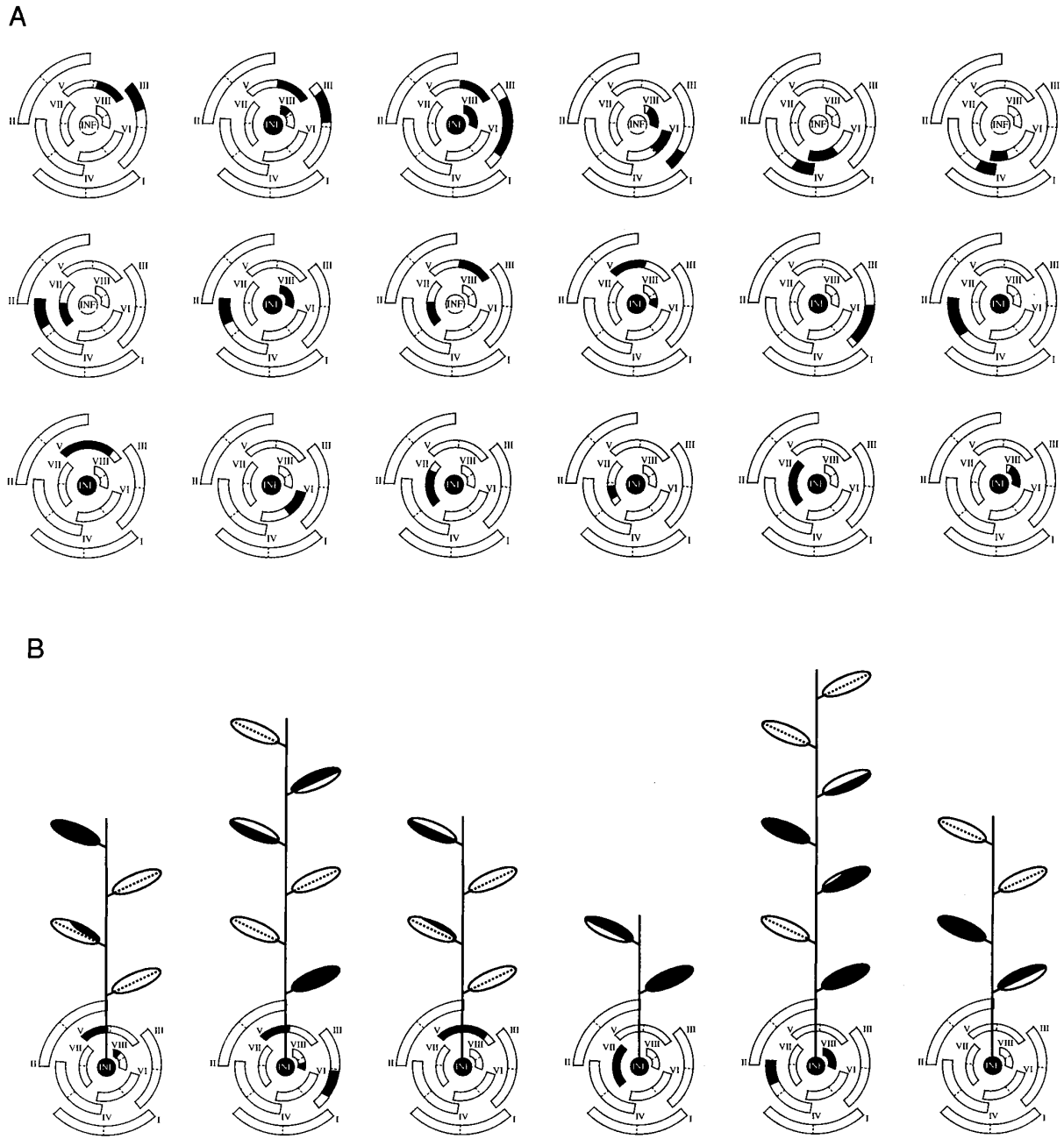


FIG. 3. Schematic illustration of multileaf sectors. The relative position of leaves is normalized to a clockwise phyllotaxy. Sectors are indicated in black. (A) Multileaf sectors of the rosette. (B) Sectors affecting the inflorescence (INF).

The relative position of precursor cells in the primary meristem and thereby the establishment of the pattern of organogenesis can be assessed by the analysis of multileaf sectors. Similar to the results previously found for L2 and L3 layers, our observations indicate that epidermal sectors (L1) result from a reproducible but variable contribution of a particular meristematic cell to late structures. Thus meristematic cells contribute to different organ primordia depending on their position within the meristem. This suggests that a patterning system is superimposed on the growth dynamics in the meristem. Evidence from studies of cytological features and cell division patterns indicates that the meristem is divided into two zones, the central zone, which shows a low cell division rate, and the periph-

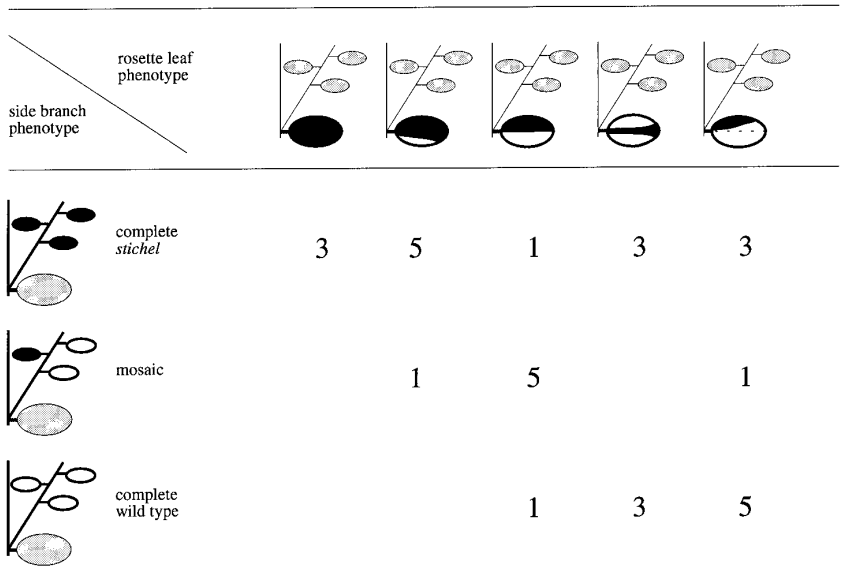


FIG. 5. Relationship between sectors on rosette leaves and the phenotype of cauline leaves from side branches. Only side branches carrying three or more leaves were used for analysis. Sectors of the rosette leaves were classified in five classes. Side branches from these rosette leaves were either *stichel,* wild type, or mosaic. The absolute numbers are shown for each class.

eral zone, which is characterized by a high proliferation rate (Wardlaw, 1957; Esau, 1977; Steeves and Sussex, 1989; Lyndon, 1990). It is thought that the pattern of organ initiation is generated in the peripheral zone (Medford, 1992). In this scenario cells adopt their fate when they are recruited to the peripheral zone. Thus the relative position of cells in the central region of the primary meristem appears to depend on a reproducible division pattern during meristem proliferation. Since progressively fewer cells of the primary meristem populate the meristem in later stages, stochastic events affecting the planes of division might become increasingly more important in determining the destination of an individual cell. This would explain the observed high variability of late sectors. In summary, our results show that fate maps of the three layers are very similar, suggesting that the meristem coordinates the growth dynamics and the patterning of the three layers.

The question remains as to what the underlying mechanism is. It is possible that the cell division pattern of one layer strictly follows that of another. This, for example, could be caused by some kind of physical constraint. Alternatively one could imagine a coordination of growth dynamics and patterning of the three layers through regulatory cellular interactions.

Some light is shed on this by our analysis of the relationship of the axillary buds with the associated leaf. It has been shown for the L2 layer that the axillary buds are derived from groups of cells located in the central portion of the leaf primordium in *Arabidopsis* (Furner and Pumfrey, 1992). The L3 was not found to contribute to axillary buds (Furner and Pumfrey, 1992). Although we observed a clonal relationship of axillary buds to the subtending leaf for the L1 layer, we were not able to find a correlation to a particular region of the associated leaf.

Two important conclusions can be drawn from the difference between L1 and L2 for the clonal relationship of axillary buds with respect to the subtending leaf. First, the data suggest that axillary meristems are initiated by L2 tissues. In addition, a model for the coordination of the three layers based on growth constraints between the layers appears to be unlikely. Thus one has to postulate regulatory interactions to coordinate proliferation of all three layers in the meristem.

ACKNOWLEDGMENTS

We thank Gerd Jürgens for his substantial support, discussion, and help. We thank M. Grebe, G. Kienzlen, B. Moussian, and K. Schrick for critically reading the manuscript. This work was supported by a Leibniz award to Gerd Jürgens from the Deutsche Forschungsgemeinschaft.

REFERENCES

Barton, M. K., and Poethig, R. S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana:* An analysis of development in the wild type and in the shoot *meristemless* mutant. *Development* 119, 823–831.

Carpenter, R., and Coen, E. S. (1990). Floral homeotic mutations

produced by transposon mutagenesis in *Antirrhinum majus*. *Genes Dev*. 4, 1483–1493.

Coe, E. H., and Neuffer, M. G. (1978). Embryo cells and their destinies in the corn plant. *In* ''The Clonal Basis of Development'' (S. Subtelny and I. M. Sussex, Eds.), pp. 113–129. Academic Press, New York.

Dolan, L., and Poethig, R. S. (1991). Genetic analysis of leaf development in cotton. *Development Suppl.* 1, 39–46.

Esau, K. (1977). ''Anatomy of Seed Plants,'' 2nd ed. Wiley, New York.

Furner, I. J., and Pumfrey, J. E. (1992). Cell fate in the shoot apical meristem of *Arabidopsis thaliana*. *Development* 115, 755–764.

Gaul, H. (1959). Über die Chimärenbildung in Gerstenpflanzen nach Röntgenbestrahlung von Samen. *Flora* 147, 207–241.

Gaul, H. (1961). Studies on diplontic selection after X-irradiation of barley seeds. *In* ''Effects of Ionizing Radiation on Seeds,'' pp. 117–138. IAEA, Vienna.

Gudkov, I. N., and Grodzinsky, D. M. (1982). Cell radiosensitivity variation in synchronously dividing root meristems of *Pisum sativum* L. and *Zea mays* L. during the mitotic cycle. *Int. J. Rad. Biol.* 41, 401–409.

Hotta, Y., and Benzer, S. (1972). Mapping of behaviour in *Drosophila* mosaics. *Nature* 240, 527–535.

Hülskamp, M., Misera, S., and Jürgens, G. (1994). Genetic dissection of trichome cell development in *Arabidopsis*. *Cell* 76, 555–566.

Irish, V. F. (1993). Cell fate determination in plant development. *Semin. Dev. Biol.* 4, 73–81.

Irish, V. F., and Sussex, I. M. (1992). A fate map of the *Arabidopsis* embryonic shoot apical meristem. *Development* 115, 745–753.

Jegla, D. E., and Sussex, I. M. (1989). Cell lineage patterns in the shoot meristem of the sunflower. *Dev. Biol.* 131, 215–225.

Johri, M. M., and Coe, E. H. (1983). Clonal analysis of corn plant development I. The development of the tassel and ear shoot. *Dev. Biol.* 97, 154–172.

Jürgens, G., and Mayer, U. (1994). Arabidopsis. *In* ''Embryos. Color Atlas of Development'' (J. B. L. Bard, Ed.), pp. 7–21. Wolfe, London.

Koornneef, M., Dellaert, L. W. M., and Van der Veen, J. H. (1982). EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana*. *Mut. Res.* 93, 109–123.

Kowyama, Y., Kawase, T., and Yamagata, H. (1984). Cell cycle dependence of radiosensitivity and mutagenesis in fertilized egg cells of rice, *Oryza sativa* L. *Theor. Appl. Genet.* 68, 297–303.

Lyndon, R. F. (1990). ''Plant Development: The Cellular Basis.'' Unwin Hyman, Winchester, MA.

Mansfield, S. G., and Briarty, L. G. (1991). Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* 69, 461–476.

Mayer, U., Ruiz, R. A. T., Berleth, T., Misera, S., and Jürgens, G. (1991). Mutations affecting body organization in the *Arabidopsis* embryo. *Nature* 353, 402–407.

McDaniel, C. N., and Poethig, R. S. (1988). Cell lineage patterns in the shoot apical meristem of the germinating maize embryo. *Planta* 175, 13–22.

Medford, J. I. (1992). Vegetative apical meristems. *Plant Cell* 4, 1029–1039.

Poethig, R. S. (1987). Clonal analysis of cell lineage patterns in plant development. *Am. J. Bot.* 74, 581–594.

Poethig, R. S., and Sussex, I. M. (1985a). The cellular parameters of leaf development in tobacco: A clonal analysis. *Planta* 165, 170–184.

Poethig, R. S., and Sussex, I. M. (1985b). The developmental morphology and growth dynamics of the tobacco leaf. *Planta* 165, 158–169.

Satina, S., Blakeslee, A. F., and Avery, A. (1940). Demonstration of three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. *Am. J. Bot.* 27, 895–905.

Scott, D., and Evans, H. J. (1967). X-ray-induced chromosomal aberrations in *Vica faba:* Change in response during the cell cycle. *Mut. Res.* 4, 579–599.

Steeves, T. A., and Sussex, I. M. (1989). ''Patterns in Plant Development,'' 2nd ed. Cambridge Univ. Press, New York.

Steffensen, D. M. (1968). A reconstruction of cell development in the shoot apex of maize. *Am. J. Bot.* 55, 354–369.

Stewart, R. N., and Burk, L. G. (1970). Independence of tissues derived from apical layers in ontogeny of the tobacco leaf and ovary. *Am. J. Bot.* 57, 1010–1016.

Stewart, R. N., Meyer, F. G., and Dermen, H. (1972). Camellia-'Daisy Eagleton,' a graft chimera of *Camellia sasanqua* and *C. japonica*. *Am. J. Bot.* 59, 515–524.

Sturtevant, A. H. (1929). The claret mutant type of *Drosophila simulans:* A study of chromosome elimination and cell lineage. *Zeitschr. f. Wiss. Zool.* 135, 323–356.

Sussex, I. M. (1989). Developmental programming of the shoot meristem. *Cell* 56, 225–229.

Szymkowiak, E. J., and Sussex, I. M. (1989). Chimeric analysis of cell layer interaction during development of the flower pedicel abscission zone. *In* ''Cell Separation in Plants'' (D. J. Osborne and M. B. Jackson, Eds.), NATO-ASI Series, pp. 363–368. Springer, Berlin.

Szymkowiak, E. J., and Sussex, I. M. (1992). The internal meristem layer (L3) determines floral meristem size and carpel number in tomato periclinal chimeras. *Plant Cell* 4, 1089–1100.

Uphof, J. C. T. (1962). ''Plant hairs.'' Gebr. Bornträger, Berlin.

Wardlaw, C. W. (1957). On the organization and reactivity of the shoot apex in vascular plants. *Am. J. Bot.* 44, 176–185.

Received for publication November 28, 1995
Accepted January 5, 1996